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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) **International Patent Classification** 5 : **C07H 21/04, A61K 48/00** — **A1**

(11) **International Publication Number:** **WO 94/21664**

(43) **International Publication Date:** 29 September 1994 (29.09.94)

(21) **International Application Number:** PCT/US94/03206

(22) **International Filing Date:** 24 March 1994 (24.03.94)

(30) **Priority Data:**
08/037,025 25 March 1993 (25.03.93) US

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(81) **Designated States:** AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) **Title:** ANTISENSE MOLECULES DIRECTED AGAINST A TENASCIN GENE

(57) **Abstract**

The present invention is directed to a polynucleotide of less than about 50 nucleic acid bases in length, which polynucleotide hybridizes to the tenascin gene. The present invention is also directed to a pharmaceutical composition comprising the above polynucleotide dissolved or dispersed in a physiologically tolerable diluent. The present invention is further directed to a process for inhibiting vascular smooth muscle cell proliferation which process comprises inhibiting the expression of tenascin in vascular smooth muscle cells.

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ANTISENSE MOLECULES DIRECTED AGAINST A TENASCIN GENE

DESCRIPTION

Cross Reference to Related Application

This application is a continuation-in-part of U.S. Application Serial No. 08/037,025, filed March 25, 1993, the disclosure of which is incorporated herein by reference.

Technical Field of the Invention

The present invention relates in general to antisense polynucleotides directed against portions of a tenascin gene or mRNA that encode tenascin. The present invention also relates to the use of such antisense polynucleotides in inhibiting the proliferation of smooth muscle cells.

Background of the Invention

Tenascin (TN) is an extracellular matrix glycoprotein consisting of six disulfide-linked subunits each of which subunit has a molecular mass of 190 to 250 kilodaltons. The expression of TN is spatially and temporally restricted, and is considered to be important for tissue interaction during physiological and pathological development.

Tenascin, which is absent in normal adult rat arterial media, was shown to appear in the neointima formed by proliferating smooth muscle cells two weeks after balloon injury of the carotid artery. Hedin et al., Am. J. Path. 139:649-656 (1991). Although the role of TN in vascular injury-related events is not well understood, it may be important for smooth muscle cell proliferation because TN has growth stimulatory activity. Chiquet-Ehrismann et al., FASEB J. 4:2598-2604 (1990).

A disease state involving the proliferation of vascular smooth muscle cells include, but not limited to, vascular stenosis, post-angioplasty restenosis (including coronary, carotid and peripheral stenosis), other non-angioplasty reopening procedures such as atherectomy and laser procedures, atherosclerosis, atrial-venous shunt failure, cardiac hypertrophy, vascular surgery, coronary artery bypass graft and organ transplant.

Activated smooth muscle cells elaborate growth factors such as platelet derived growth factor (PDGF), basic and acidic fibroblast growth factor, interleukins and transforming growth factor β. Likewise, the smooth muscle cells increase the production of PDGF receptor, FGF receptor, and epidermal growth factor receptor.

Activation of smooth muscle cells, leading to the proliferation of those cells, occurs in response to a number of stimuli, including surgical procedures such as coronary angioplasty. The proliferation of smooth muscle cells results in such disease states as atherosclerosis and restenosis.

An in vitro assay system has been developed to study smooth muscle cell proliferation. This assay system is considered to be a useful model for smooth muscle cell proliferation in vivo. Gordon et al. have shown that smooth muscle cell proliferation results from aortic and carotid balloon catheter injury, and is a result of atherosclerosis, providing a positive correlation between smooth muscle cell proliferation and stenosis. Gordon et al., Proc. Natl. Acad. Sci. USA 87:4600-4604 (1990).

Speir et al. have studied the inhibition of smooth muscle cell proliferation in vitro by using an antisense oligonucleotide to proliferating cell nuclear antigen (PCNA). However, these workers could not inhibit

proliferation below 50 %, and the inhibition required high levels of the 18-mer antisense oligonucleotide used in those studies.

This invention demonstrates the biological action of antisense polynucleotides directed against the tenascin gene, as useful for anti-proliferative activity against smooth muscle cell proliferation. This invention is applicable to a number of disease states in which the proliferation of smooth muscle cells is involved, including, but not limited to, vascular stenosis, post-angioplasty restenosis (including coronary, carotid and peripheral stenosis), other non-angioplasty reopening procedures such as atherectomy and laser procedures, atherosclerosis, atrial-venous shunt failure, cardiac hypertrophy, vascular surgery, and organ transplant.

Brief Summary of the Invention

In one aspect, the present invention provides a synthetic antisense polynucleotide of less than about 50 bases, preferably less than about 35 bases, more preferably less than about 25 bases, and most preferably less than about 20 bases, comprising a nucleotide sequence that is identical to at least 18 contiguous bases of TGGCARYAGC YRRGTCAYGG CCCCCATGGT GGAGGT (SEQ ID NO:1), where R is A or G, and Y is C or T.

In a preferred embodiment, the antisense polynucleotide is a polydeoxyribonucleotide which comprises the nucleotide sequence CYRRGTCAYG GCCCCCAT (SEQ ID NO:3), where R is A or G and Y is T or C.

In another preferred embodiment, the antisense polynucleotide is a polyribonucleotide which comprises the nucleotide sequence

CYRRGUCAYG GCCCCCAU (SEQ ID NO:5), where R is A or G and Y is U or C.

In another aspect, the present invention provides a synthetic antisense polynucleotide of less than about 50 bases, preferably less than about 35 bases, more preferably less than about 25 bases, and most preferably less than about 20 bases, comprising a nucleotide sequence that is identical to at least 18 contiguous bases of ACCTCCACCA TGGGGGCCRT GACYYRGCTR YTGCCA (SEQ ID NO:2), where R is A or G, and Y is C or U.

In a preferred embodiment, the antisense polynucleotide is a polydeoxyribonucleotide which comprises the nucleotide sequence ATGGGGGCCR TGACYYRG (SEQ ID NO:4), where R is A or G and Y is T or C.

In another preferred embodiment, the antisense polynucleotide is a polyribonucleotide which comprises the nucleotide sequence AUGGGGGCCR UGACYYRG (SEQ ID NO:6), where R is A or G and Y is U or C.

Preferably, the bases of a polynucleotide of the present invention are linked by pseudophosphate bonds that are resistant to cleavage by exonuclease or endonuclease enzymes and, more preferably the pseudophosphate bonds are phosphorothioate bonds.

In another aspect, the present invention provides a pharmaceutical composition comprising a polynucleotide of the present invention and a physiologically tolerable diluent.

In yet another aspect, the present invention provides a process of inhibiting vascular smooth muscle cell proliferation comprising inhibiting the expression of tenascin in the vascular smooth muscle cell.

In one embodiment of that process, the expression of tenascin is inhibited by inhibiting the transcription of the gene that encodes tenascin. Inhibition of transcription of the gene that encodes tenascin is preferably accomplished by exposing the smooth muscle cell to an antisense polydeoxyribonucleotide of the present invention. Preferred such polydeoxyribonucleotides are set forth above.

In another embodiment of the process of this invention, the expression of tenascin is inhibited by inhibiting the translation of mRNA that encodes tenascin. Preferably, inhibition of mRNA translation is accomplished by exposing the smooth muscle cell to an antisense polydeoxyribonucleotide of the present invention. Preferred such polydeoxyribonucleotides are set forth above.

Brief Description of the Invention

In the drawings, which form a portion of the specification:

Figure 1 shows the percentage of growth inhibition of smooth muscle cells upon the addition of various concentrations of antisense polynucleotides directed against the tenascin gene.

Figure 2 shows the percentage of maximal intimal thickening of rat carotid artery upon the addition of antisense polynucleotides to tenascin or plasminogen activator inhibitor-1 (PAI1), or to a control solution containing the carrier or vehicle only.

Detailed Description of the Invention

I. The Invention

The present invention provides antisense polynucleotides directed against polynucleotide sequences that encode tenascin. As used herein, "polynucleotide" refers to a covalently linked sequence of nucleotides in which the 3' position of the pentose of one nucleotide is joined by a phosphodiester or pseudophosphate group to the 5' position of the pentose of the next nucleotide. An antisense polynucleotide of the present invention can be a polydeoxyribonucleotide comprising the bases found in DNA (A, G, T and C), or a polyribonucleotide comprising the bases found in RNA (A, G, U and C).

An antisense polynucleotide of the present invention is directed against a target polynucleotide sequence that encodes tenascin. The target polynucleotide sequence encoding tenascin can be a DNA sequence, such as a genomic or cDNA sequence, or an RNA sequence such as an mRNA molecule encoding tenascin.

Preferably, an antisense polynucleotide of the present invention is directed against a portion of a polynucleotide that encodes tenascin. Preferably, that target portion flanks the mRNA initiation site (the start codon, ATG for methionine). In an especially preferred embodiment, an antisense polynucleotide of the present invention is directed against a portion from about 10 base positions upstream to about 30 base positions downstream from the mRNA initiation site.

Amino acid residue sequences for tenascin and polynucleotide sequences that encode tenascin have been described for a number of animal species including chicken (Spring et al., Cell 59:325-334, 1989), rodent (Saga et al., Gene 104:177-185, 1991), pig (Nishi et al. Eur. J. Biochem. 202:643-

648, 1991) and human (Siri et al., Nuc. Acids Res. 19:525-531, 1991). While there are recognized differences among those species in portions of the amino acid residue and encoding polynucleotide sequences for tenascin, there is a high degree of sequence homology among species in 1) the amino-terminal region of the amino acid residue sequence and 2) genomic DNA sequences flanking the mRNA initiation site. Based on known DNA sequences encoding tenascin, a consensus sequence around the mRNA initiation site can be derived. Such a consensus sequence is presented below.

ACCUCCACCA UGGGGGCCRU GACYYRGCUR YUGCCA (SEQ ID NO:63) , where R is A or G, and Y is C or U. The mRNA start site, AUG, is shown in bold.

Based on this consensus mRNA sequence, a consensus sequence for the DNA encoding this mRNA (i.e., a tenascin gene consensus sequence) can be derived. Such a consensus sequence is presented below.

TGGCARYAGC YRRGTCAYGG CCCCCATGGT GGAGGT (SEQ ID NO:64) where R is A or G, and Y is C or T.

The present invention also provides a use of an antisense polynucleotide of this invention. The present inventors have discovered that the antisense molecules directed against either an RNA or DNA molecule encoding tenascin can be used to inhibit the expression of tenascin, which inhibition of tenascin expression results in an inhibition of proliferation of smooth muscle cells. Tenascin expression can be inhibited by inhibiting either transcription of the gene encoding tenascin or by inhibiting translation of tenascin from mRNA.

Antisense polynucleotides contain sequences of nucleotide bases complementary to messenger RNA (mRNA or message) or the sense strand of double stranded DNA (i.e., a gene). Admixture of sense and antisense oligo- or polynucleotides leads to binding or hybridization of the two molecules.

When antisense polynucleotides hybridize with mRNA, inhibition of translation occurs. When these antisense polynucleotides bind to double stranded DNA, inhibition of transcription occurs. The resulting inhibition of translation and/or transcription leads to an inhibition of the synthesis of the encoded protein.

As is well known in the art, polynucleotide hybridization is a function of sequence identity, G+C content of the sequence, buffer salt content, sequence length and duplex melt temperature (T_m) among other variables. See, Maniatis et al., Molecular Cloning, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1982), page 388.

With similar sequence lengths, the buffer salt concentration and temperature provide useful variables for assessing sequence identity by hybridization techniques. For example, where there is at least 90 percent identity, hybridization is carried out at 68°C in a buffer salt such as 6XSCC diluted from 20XSSC [Maniatis et al., above, at page 447]. The buffer salt utilized for final Southern blot washes can be used at a low concentration, e.g., 0.1XSSC and at a relatively high temperature, e.g. 68°C, and two sequences will form a hybrid duplex (hybridize). Use of the above hybridization and washing conditions together are defined as conditions of high stringency or highly stringent conditions.

Moderately high stringency conditions can be utilized for hybridization where two sequences share at least about 80 percent identity. Here, hybridization is carried out using 6XSSC at a temperature of about 50-55°C. A final wash salt concentration of about 1-3XSSC and at a temperature of about 60-68°C are used. These hybridization and washing conditions define moderately high stringency conditions.

Low stringency conditions can be utilized for hybridization where two sequences share at least 40 percent identity. Here, hybridization carried out using 6XSSC at a temperature of about 40-50°C, and a final wash buffer salt concentration of about 6XSSC used at a temperature of about 40-60°C effect non-random hybridization. These hybridization and washing conditions define low stringency conditions.

Polynucleotide sequence information provided by the present invention allows for the preparation of antisense polynucleotides that hybridize to sequences of the tenascin gene or mRNA disclosed herein. In these aspects, antisense polynucleotides of an appropriate length are prepared based on a consideration of a selected nucleotide sequence, e.g., a sequence such as that shown in SEQ ID NO:1 or SEQ ID NO:2. The ability of such nucleic acid probes to specifically hybridize to the tenascin gene or mRNA lends them particular utility in a variety of embodiments.

A. Antisense Polynucleotides

In one aspect, the present invention provides a synthetic antisense polynucleotide of less than about 50 bases, preferably less than about 35 bases, more preferably less than about 25 bases, and most preferably less than about 20 bases, comprising a nucleotide sequence that is identical to at least 18 contiguous bases of TGGCARYAGC

YRRGTCAYGG CCCCCATGGT GGAGGT (SEQ ID NO:1) where R is A or G, and Y is C or T.

In a preferred embodiment, the antisense polynucleotide is a polydeoxyribonucleotide which comprises the nucleotide sequence CYRRGTCAYG GCCCCCAT (SEQ ID NO:3), where R is A or G and Y is T or C. In an especially preferred embodiment, the polydeoxyribonucleotide has the nucleotide sequence of SEQ ID NOs:7-19 or 20.

The following especially preferred polydeoxyribonucleotides which polydeoxyribonucleotides are directed against the consensus mRNA sequence of tenascin shown in SEQ ID NO:63.

CCAGGTCACG GCCCCCAT (SEQ ID NO:7) is directed against the sequence from +1 to +18 of SEQ ID NO:2.

GGCCCCCATG GTGGAGGT (SEQ ID NO:8) is directed against the sequence from -9 to +9 of SEQ ID NO:2.

TGGCAGTAGC CAGGTCAC (SEQ ID NO:9) is directed against the sequence from +10 to +27 of SEQ ID NO:2.

TAGCCAGGTC ACGGCCCCCA T (SEQ ID NO:10) is directed against the sequence from +1 to +21 of SEQ ID NO:2.

CAGTAGCCAG GTCACGGCCC CCAT (SEQ ID NO:11) is directed against the sequence from +1 to +24 of SEQ ID NO:2.

CCAGGTCACG GCCCCCATGG T (SEQ ID NO:12) is directed against the sequence from -3 to +18 of SEQ ID NO:2.

TAGCCAGGTC ACGGCCCCCA TGGT (SEQ ID NO:13) is directed against the sequence from -3 to +21 of SEQ ID NO:2.

CTGAGTCATG GCCCCCAT (SEQ ID NO:14) is directed against the sequence from +1 to +18 of SEQ ID NO:2.

CAGCTGAGTC ATGGCCCCCA TGGT (SEQ ID NO:15) is directed against the sequence from -3 to +21 of SEQ ID NO:2.

CTGAGTCATG GCCCCCATGG T (SEQ ID NO:16) is directed against the sequence from -3 to +18 of SEQ ID NO:2.

CAACAGCTGA GTCATGGCCC CCAT (SEQ ID NO:17) is directed against the sequence from +1 to +24 of SEQ ID NO:2.

CAGCTGAGTC ATGGCCCCCA T (SEQ ID NO:18) is directed against the sequence from +1 to +21 of SEQ ID NO:2.

CTGAGTCATG GCCCCCATGG TGGA (SEQ ID NO:19) is directed against the sequence from -6 to +18 of SEQ ID NO:2.

CAACAGCTGA GTCATGGCCC CCATGGT (SEQ ID NO:20) is directed against the sequence from -3 to +24 of SEQ ID NO:2.

In another preferred embodiment, the antisense polynucleotide is a polyribonucleotide which comprises the nucleotide sequence CYRRGUCAYG GCCCCCAU (SEQ ID NO:5), where R is A or G and Y is U or C. In an especially preferred embodiment, the polyribonucleotide has a nucleotide sequence selected from the group consisting of SEQ ID NOs:35-47 or 48.

The following are especially preferred polyribonucleotides which polyribonucleotides are directed against the consensus mRNA for tenascin shown in SEQ ID NO:63.

CCAGGUCACG GCCCCCAU (SEQ ID NO:35) is directed against the sequence from +1 to +18 of SEQ ID NO:2.

GGCCCCCAUG GUGGAGGU (SEQ ID NO:36) is directed against the sequence from -9 to +9 of SEQ ID NO:2.

UGGCAGUAGC CAGGUCAC (SEQ ID NO:37) is directed against the sequence from +10 to +27 of SEQ ID NO:2.

UAGCCAGGUC ACGGCCCCCA U (SEQ ID NO:38) is directed against the sequence from +1 to +21 of SEQ ID NO:2.

CAGUAGCCAG GUCACGGCCC CCAU (SEQ ID NO:39) is directed against the sequence from +1 to +24 of SEQ ID NO:2.

CCAGGUCACG GCCCCCAUGG U (SEQ ID NO:40) is directed against the sequence from -3 to +18 of SEQ ID NO:2.

UAGCCAGGUC ACGGCCCCCA UGGU (SEQ ID NO:41) is directed against the sequence from -3 to +21 of SEQ ID NO:2.

CUGAGUCAUG GCCCCCAU (SEQ ID NO:42) is directed against the sequence from +1 to +18 of SEQ ID NO:2.

CAGCUGAGUC AUGGCCCCCA UGGU (SEQ ID NO:43) is directed against the sequence from -3 to +21 of SEQ ID NO:2.

CUGAGUCAUG GCCCCCAUGG U (SEQ ID NO:44) is directed against the sequence from -3 to +18 of SEQ ID NO:2.

CAACAGCUGA GUCAUGGCCC CCAU (SEQ ID NO:45) is directed against the sequence from +1 to +24 of SEQ ID NO:2.

CAGCUGAGUC AUGGCCCCCA U (SEQ ID NO:46) is directed against the sequence from +1 to +21 of SEQ ID NO:2.

CUGAGUCAUG GCCCCCAUGG UGGA (SEQ ID NO:47) is directed against the sequence from -6 to +18 of SEQ ID NO:2.

CAACAGCUGA GUCAUGGCCC CCAUGGU (SEQ ID NO:48) is directed against the sequence from -3 to +24 of SEQ ID NO:2.

In another aspect, the present invention provides a synthetic antisense polynucleotide of less than about 50 bases, preferably less than about 35 bases, more preferably less than about 25 bases, and most preferably less than about 20 bases, comprising a nucleotide sequence that is identical to at least 18 contiguous bases of ACCTCCACCA TGGGGGCCRT GACYYRGCTR YTGCCA (SEQ ID NO:2), where R is A or G, and Y is C or U.

In a preferred embodiment, the antisense polynucleotide is a polydeoxyribonucleotide which comprises the nucleotide sequence ATGGGGGCCR TGACYYRG (SEQ ID NO:4), where R is A or G and Y is T or C. In an especially preferred embodiment, the polydeoxyribonucleotide has the nucleotide sequence of SEQ ID NO:21-33 or 34.

The following especially preferred polydeoxyribonucleotides which polydeoxyribonucleotides are directed against the consensus gene for tenascin shown in SEQ ID NO:64.

ATGGGGGCCGTGACCTGG (SEQ ID NO:21) is directed against the sense strand of the gene for tenascin coding the mRNA from +1 to +18 of SEQ ID NO:1.

ACCTCCACCATGGGGGCC (SEQ ID NO:22) is directed against the sense strand of the gene coding for the mRNA from -9 to +9 of SEQ ID NO:1.

GTGACCTGGCTACTGCCA (SEQ ID NO:23) is directed against the sense strand of the gene coding for the mRNA from +10 to +27 of SEQ ID NO:1.

ATGGGGGCCGTGACCTGGCTA (SEQ ID NO:24), directed against the sense strand of the gene coding for the mRNA from +1 to +21 of SEQ ID NO:1.

ATGGGGGCCGTGACCTGGCTACTG (SEQ ID NO:25) is directed against the sense strand of the gene coding for the mRNA from +1 to +24 of SEQ ID NO:1.

ACCATGGGGGCCGTGACCTGG (SEQ ID NO:26) is directed against the sense strand of the gene coding for the mRNA from -3 to +18 of SEQ ID NO:1.

ACCATGGGGGCCGTGACCTGGCTA (SEQ ID NO:27) is directed against the sense strand of the gene coding for the mRNA from -3 to +21 of SEQ ID NO:1.

ATGGGGGCCATGACTCAG (SEQ ID NO:28) is directed against the sense strand of the gene coding for the mRNA from +1 to +18 of SEQ ID NO:1.

ACCATGGGGGCCATGACTCAGCTG (SEQ ID NO:29) is directed against the sense strand of the gene coding for the mRNA from -3 to +21 of SEQ ID NO:1.

ACCATGGGGGCCATGACTCAG (SEQ ID NO:30) is directed against the sense strand of the gene coding for the mRNA from -3 to +18 of SEQ ID NO:1.

ATGGGGGCCATGACTCAGCTGTTG (SEQ ID NO:31) is directed against the sense strand of the gene coding for the mRNA from +1 to +24 of SEQ ID NO:1.

ATGGGGGCCATGACTCAGCTG (SEQ ID NO:32) is directed against the sense strand of the gene coding for the mRNA from +1 to +21 of SEQ ID NO:1.

TCCACCATGGGGGCCATGACTCAG (SEQ ID NO:33) is directed against the sense strand of the gene coding for the mRNA from -6 to +18 of SEQ ID NO:1.

ACCATGGGGGCCATGACTCAGCTGTTG (SEQ ID NO:34) is directed against the sense strand of the gene coding for the mRNA from -3 to +24 of SEQ ID NO:1.

In another preferred embodiment, the antisense polynucleotide is a polyribonucleotide which comprises the nucleotide sequence AUGCCCCGGR UGACYYRG (SEQ ID NO:6), where R is A or G and Y is U or C. In an especially preferred embodiment, the polyribonucleotide has the nucleotide sequence of SEQ ID NO:49-61 or 62.

The following especially preferred polyribonucleotides which polyribonucleotides are directed against the consensus gene for tenascin shown in SEQ ID NO:64.

AUGGGGGCCGUGACCUGG (SEQ ID NO:49) is directed against the sense strand of the gene for tenascin coding the mRNA from +1 to +18 of SEQ ID NO:1.

ACCUCCACCAUGGGGGCC (SEQ ID NO:50) is directed against the sense strand of the gene coding for the mRNA from -9 to +9 of SEQ ID NO:1.

GUGACCUGGCUACUGCCA (SEQ ID NO:51) is directed against the sense strand of the gene coding for the mRNA from +10 to +27 of SEQ ID NO:1.

AUGGGGGCCGUGACCUGGCUA (SEQ ID NO:52), directed against the sense strand of the gene coding for the mRNA from +1 to +21 of SEQ ID NO:1.

AUGGGGGCCGUGACCUGGCUACUG (SEQ ID NO:53) is directed against the sense strand of the gene coding for the mRNA from +1 to +24 of SEQ ID NO:1.

ACCAUGGGGGCCGUGACCUGG (SEQ ID NO:54) is directed against the sense strand of the gene coding for the mRNA from -3 to +18 of SEQ ID NO:1.

ACCAUGGGGGCCGUGACCUGGCUA (SEQ ID NO:55) is directed against the sense strand of the gene coding for the mRNA from -3 to +21 of SEQ ID NO:1.

AUGGGGGCCAUGACUCUG (SEQ ID NO:56) is directed against the sense strand of the gene coding for the mRNA from +1 to +18 of SEQ ID NO:1.

ACCAUGGGGGCCAUGACUCAGCUG (SEQ ID NO:57) is directed against the sense strand of the gene coding for the mRNA from -3 to +21 of SEQ ID NO:1.

ACCAUGGGGGCCAUGACUCAG (SEQ ID NO:58) is directed against the sense strand of the gene coding for the mRNA from -3 to +18 of SEQ ID NO:1.

AUGGGGGCCAUGACUCAGCUGUUG (SEQ ID NO:59) is directed against the sense strand of the gene coding for the mRNA from +1 to +24 of SEQ ID NO:1.

AUGGGGGCCAUGACUCAGCUG (SEQ ID NO:60) is directed against the sense strand of the gene coding for the mRNA from +1 to +21 of SEQ ID NO:1.

UCCACCAUGGGGGCCAUGACUCAG (SEQ ID NO:61) is directed against the sense strand of the gene coding for the mRNA from -6 to +18 of SEQ ID NO:1.

ACCAUGGGGGCCAUGACUCAGCUGUUG (SEQ ID NO:62) is directed against the sense strand of the gene coding for the mRNA from -3 to +24 of SEQ ID NO:1.

It is to be understood that the present invention further contemplates antisense polynucleotides that hybridizes to any gene encoding an isoform of tenascin. Any such polynucleotide capable of inhibiting the proliferation of smooth muscle cell proliferation can be used.

The position numbers of the sequences listed herein are all relative to the first nucleic acid base of the mRNA start site, which is denoted +1. Thus, the initial adenine of the ATG start codon of the mRNA is given the position +1; nucleic acid bases in the coding region of the mRNA are given positive values relative to +1, while nucleic acid bases

that are in the non-coding region in the 5' direction from the start codon are given negative values.

In the case of the polynucleotides listed above, since these polynucleotides are complementary to the mRNA of tenascin, the +1 position is a thymine, which is the complementary base to the adenine of the mRNA start site.

Preferably, the bases of a polynucleotide of the present invention are linked by pseudophosphate bonds that are resistant to cleavage by exonuclease or endonuclease enzymes and, more preferably the pseudophosphate bonds are phosphorothioate bonds.

Exonuclease enzymes hydrolyze the terminal phosphodiester bond of a nucleic acid. Endonuclease enzymes hydrolyze internal phosphodiester bonds of a nucleic acid.

By replacing a phosphodiester bond with one that is resistant to the action of exonucleases or endonucleases, the stability of the nucleic acid in the presence of those exonucleases or endonucleases is increased. As used herein, pseudophosphate bonds include, but are not limited to, methylphosphonate, phosphomorpholidate, phosphorothioate, phosphorodithioate and phosphoroselenoate bonds. Additionally, exonuclease and/or endonuclease resistant polynucleotides can be obtained by blocking the 3' and/or 5' terminal nucleotides with substituent groups such as acridine or cholesterol.

Preferred pseudophosphate bonds are phosphorothioate bonds. The pseudophosphate bonds may comprise the bonds at the 3' and or 5' terminus, the bonds from about 1 to about 5 of the 3' and/or 5' terminus

bases, or the bonds of the entire polynucleotide. A preferred polynucleotide with pseudophosphate bonds is one in which all of the bonds are comprised of pseudophosphate bonds. A further preferred polynucleotide with pseudophosphate bonds is one in which the polynucleotide has mixed phosphorothioate and phosphodiester bonds, that is, a polynucleotide in which the about 5 bonds of the 3' and 5' termini are pseudophosphate bonds (e.g., phosphorothioate bonds) and the remaining bonds are phosphodiester bonds.

DNA or RNA polynucleotides can be prepared using several different methods, as is well known in the art. See, e.g., Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, New York (1990). The phosphoramidate synthesis method is described in Caruthers et al., Meth. Enzymol. 154:287 (1987); the phosphorothioate polynucleotide synthesis method is described in Iyer et al., J. Am. Chem. Soc. 112:1253 (1990).

A preferred method of polydeoxyribonucleotide synthesis is via cyanoethyl phosphoramidite chemistry. Antisense polydeoxyribonucleotide solid phase syntheses can be performed on columns using cyanoethyl phosphoramidite chemistry on DNA synthesizer replacing iodine by 3H-1,2-benzodithiol-3-one 1,1-dioxide (BDTD Beaucage reagent). The polynucleotide is cleaved from the solid support by incubation with concentrated ammonium hydroxide.

The solution is collected and deprotected. The contents are transferred to a glass tube, chilled on ice and evaporated to dryness.

The polynucleotide is then dissolved in triethylammonium acetate (TEAA). The polynucleotide is detritylated and purified. This

procedure, first separates the trityl-on full length polynucleotide from its failure sequences containing free hydroxyl groups and synthesis reagents. This is followed by the removal of 5'-DMT by 0.5% TFA. Finally the gradient resolves the desired detritylated sequence from other contaminants.

Absorbance is monitored at 260 nm to identify factions containing the polynucleotide which is then evaporated. The polynucleotide is dissolved in water, evaporated to remove volatile salts, and finally dissolved in 0.5 ml sterile, low TE (10mM Tris, 1mM EDTA, pH 7.5).

The polynucleotide concentration is determined by measuring the absorbance at 260 nm. Typical yields are 30-40%. The integrity of the polynucleotide is determined by polyacrylamide gel electrophoresis (PAGE; 20% polyacrylamide, 7M urea) and staining with 0.2% methylene blue.

The polynucleotides used in the Examples presented herein were prepared using the polynucleotide synthesis method discussed above.

In another aspect, the present invention provides a pharmaceutical composition comprising a polynucleotide of the present invention and a physiologically tolerable diluent.

The present invention includes one or more polynucleotides as described above formulated into compositions together with one or more non-toxic physiologically tolerable or acceptable diluents, carriers, adjuvants or vehicles that are collectively referred to herein as diluents, for parenteral injection, for oral administration in solid or liquid form, for rectal or topical administration, or the like.

The compositions can be administered to humans and animals either orally, rectally, parenterally (intravenous, by intramuscularly or subcutaneously), intracisternally, intravaginally, intraperitoneally, locally (powders, ointments or drops), or as a buccal or nasal spray.

The compositions can also be delivered through a catheter for local delivery at the site of vascular damage, via an intracoronary stent (a tubular device composed of a fine wire mesh), or via a biodegradable polymer. The compositions may also be complexed to ligands, such as antibodies, for targeted delivery of the compositions to the site of smooth muscle cell proliferation.

The compositions are preferably administered via parenteral delivery at the local site of smooth muscle cell proliferation. The parenteral delivery is preferably via catheter.

Compositions suitable for parenteral injection may comprise physiologically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions and sterile powders for reconstitution into sterile injectable solutions or dispersions. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (propyleneglycol, polyethyleneglycol, glycerol, and the like), suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants.

These compositions can also contain adjuvants such as preserving, wetting, emulsifying, and dispensing agents. Prevention of the

action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. It may also be desirable to include isotonic agents, for example sugars, sodium chloride and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin.

Besides such inert diluents, the composition can also include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening, flavoring and perfuming agents.

Suspensions, in addition to the active compounds, may contain suspending agents, as for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, or mixtures of these substances, and the like.

Dosage forms for topical administration of a compound of this invention include ointments, powders, sprays and inhalants. The active component is admixed under sterile conditions with a physiologically acceptable carrier and any preservatives, buffers or propellants as may be required. Ophthalmic formulations, eye ointments, powders and solutions are also contemplated as being within the scope of this invention.

The agents can also be administered in the form of liposomes. As is known in the art, liposomes are generally derived from phospholipids or other lipid substances. Liposomes are formed by mono- or multilamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically acceptable and metabolizable lipid capable of forming liposomes can be used. The present compositions in liposome form can

contain stabilizers, preservatives, excipients, and the like in addition to the agent. The preferred lipids are phospholipids and phosphatidyl cholines (lecithins), both natural and synthetic.

Methods of forming liposomes are known in the art. See, for example, Prescott, Ed., Methods in Cell Biology, Volume XIV, Academic Press, New York, N.Y. (1976), p. 33 et seq.

The compositions of the present invention can also be delivered by viral vectors. Such vectors include adenoviral vectors, retroviral vectors and herpes simplex virus vectors, as are well known in the art. The use of such vectors largely eliminates the problems of delivery undegraded DNA or RNA to target cells in vivo and in vitro.

Actual dosage levels of active ingredients in the compositions of the present invention may be varied so as to obtain an amount of active ingredient that is effective to obtain a desired therapeutic response for a particular composition and method of administration. The selected dosage level therefore depends upon the desired therapeutic effect, on the route of administration, on the desired duration of treatment and other factors.

The total daily dose of the compounds of this invention administered to a host in single or divided dose may be in amounts, for example, of from about 1 nanomol to about 5 micromols per kilogram of body weight. Dosage unit compositions may contain such amounts of such submultiples thereof as may be used to make up the daily dose. It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the body weight, general health, sex, diet, time and route of administration, rates of absorption and

excretion, combination with other drugs and the severity of the particular disease being treated.

B. A Process of Inhibiting Vascular Smooth Muscle Cell Proliferation

In yet another aspect, the present invention provides a process of inhibiting vascular smooth muscle cell proliferation comprising inhibiting the expression of tenascin in the vascular smooth muscle cell.

In one embodiment of that process, the expression of tenascin is inhibited by inhibiting the transcription of the gene that encodes tenascin. Inhibition of transcription of the gene that encodes tenascin is preferably accomplished by exposing the smooth muscle cell to an antisense polynucleotide of the present invention. Preferred such polynucleotides are set forth above.

In another embodiment of the process of this invention, the expression of tenascin is inhibited by inhibiting the translation of mRNA that encodes tenascin. Preferably, inhibition of mRNA translation is accomplished by exposing the smooth muscle cell to an antisense polynucleotide of the present invention. Preferred such polynucleotides are set forth above.

Inhibition of transcription or translation involves hybridization between the antisense polynucleotide and the target DNA or RNA sequence. As is well known in the art, hybridization can occur between two DNA molecules, between two RNA molecules or between a DNA molecule and a RNA molecule. Thus, where the target sequence is a DNA molecule (where transcription is to be inhibited), a process of the present invention can use either an antisense polydeoxyribonucleotide (e.g., SEQ ID NOs:7-

34) or an antisense polyribonucleotide (e.g., SEQ ID NOs: 35-62). In a similar manner, where the target sequence is a RNA molecule (where translation is to be inhibited), a process of the present invention can use either an antisense polydeoxyribonucleotide or polyribonucleotide.

The selection of whether to use an antisense polydeoxyribo- or polyribo-nucleotide depends, as is well known in the art on the particular use of the antisense molecule. Thus, where the antisense polynucleotide is used in an environment having catalytic amounts of ribonucleases (RNAse) or deoxyribonucleases (DNAse), it is preferred to use an antisense polydeoxy ibonucleotide or a polyribonucleotide, respectively, to minimize degradation.

Where a particular environment is characterized by catalytic amounts of both RNAses and DNAses, a preferred antisense polynucleotide is one containing pseudophosphate bonds as set forth above, which bonds are resistant to catalytic degradation by nucleases.

Preferably, exposing the smooth muscle cells involves contacting the smooth muscle cell with the antisense polynucleotides of the present invention. Contact is achieved by admixing the polynucleotide composition with a preparation of vascular smooth muscle cells.

The amount of antisense polynucleotide of the present invention capable of inhibiting the proliferation of smooth muscle cells is an inhibition-effective amount. As used herein, an "inhibition-effective amount" is that amount of a polynucleotide of the present invention which is sufficient for inhibiting the proliferation of a cell contacted with such a polynucleotide. Means for determining an inhibition-effective amount in a particular subject will depend, as is well known in the art, on the nature of

the polynucleotide used, the mass of the subject being treated, whether killing or growth inhibition of the cells is desired, and the like.

The antisense polynucleotides useful in the process of the present invention are preferably administered under biological culture conditions. Biological culture conditions are those conditions necessary to maintain the growth and replication of the vascular smooth muscle cells in a normal, polynucleotide-free environment. These biological culture conditions, encompassing such factors as temperature, humidity, atmosphere, pH and the like, must be suitable for the proliferation of vascular smooth muscle cells in the absence of polynucleotides so that the effects of such polynucleotides on relevant growth parameters can be measured.

A preferred polynucleotide useful in this process has the sequence shown in SEQ ID NOs:3-62. A further preferred polynucleotide useful in this process links the bases of the polynucleotides shown in SEQ ID NOs:3-62 by pseudophosphate bonds that are resistant to cleavage by exonuclease enzymes. Preferred pseudophosphate bonds are phosphorothioate bonds. In a preferred embodiment, the polynucleotide as described above is dissolved or dispersed in a physiologically tolerable diluent.

It is clear from the following examples that the in vitro activity of the antisense polynucleotides of the present invention in inhibiting the proliferation of smooth muscle cells is predictive of, and correlates to, the in vivo activity of those polynucleotides in an animal model system used in studying smooth muscle cell proliferation. The rat carotid artery model of restenosis is well known in the art as an effective model system for mammalian, and particularly human, restenosis and other disease states involving the proliferation of vascular smooth muscle cells.

The following examples illustrate particular embodiments of the present invention and are not limiting of the specification and claims in any way.

EXAMPLES

Example 1: Synthesis of Antisense Polynucleotides

Fourteen antisense polynucleotides directed against mRNA molecules encoding tenascin were synthesized in accordance with standard procedures well known in the art. Seven of the antisense polynucleotides (SEQ ID NOs:7-13) were directed against rodent mRNA and seven antisense polynucleotides (SEQ ID NOs:14-20) were directed against human mRNA encoding tenascin. The antisense polydeoxynucleotides used in these Examples were protected from nucleases by replacing every phosphodiester bond with a phosphorothioate bond.

Briefly, polynucleotide synthesis was carried out using cyanoethyl phosphoramidite chemistry. Antisense polydeoxyribonucleotide solid phase syntheses was performed on Millipore CPG columns using cyanoethyl phosphoramidite chemistry on an Eppendorf Synostal D300 DNA synthesizer replacing iodine by 3H-1,2-benzodithiol-3-one 1,1-dioxide (BDTD Beaucage reagent). The polynucleotide was cleaved from the solid support by incubation with 3 ml of fresh, concentrated (30%) ammonium hydroxide for 90 minutes. Cleavage was facilitated by mixing of the solution every 30 minutes with the help of two 5 ml slip-tip syringes.

The solution was collected in a screw-capped glass vial and deprotection was accomplished either at room temperature for 24 hours or at 55 °C for 5 hours. The contents were transferred to a 13x100 mm glass tube, chilled on ice and evaporated to dryness using a Savant Speed-Vac.

The polynucleotide was then dissolved in 1 ml of 0.1M triethylammonium acetate (TEAA), pH 7.0. The polynucleotide was detritylated and purified on a Rainin Dynamax C8 semipreparative column (10mm x 25cm, 5um, 300 A). The mobile phases were (A): 0.1M TEAA, pH7.0, 5% acetonitrile; (B): 95% acetonitrile, 5% water; (C): 0.5% TFA in water. The column was developed at 2ml/min with the following gradient: 10% B in A, 10 min; 100% A, 4 min; 100% C, 8 min; 100% A, 8 min; 100% A to 45% B in 24 min. This procedure first separated the trityl-on full length polynucleotide from its failure sequences containing free hydroxyl groups and synthesis reagents. This was followed by the removal of 5'-DMT by 0.5% TFA. Finally the gradient resolved the desired detritylated sequence from other contaminants.

Absorbance was monitored at 260 nm to identify factions containing the polynucleotide which was then evaporated. The polynucleotide was dissolved in 1 ml water, evaporated to remove volatile salts, and finally dissolved in 0.5 ml sterile, low TE (10mM Tris, 1mM EDTA, pH 7.5).

The polynucleotide concentration was determined by measuring the absorbance at 260 nm. Typical yields were 30-40%. The integrity of the polynucleotide was determined by polyacrylamide gel electrophoresis (PAGE; 20% polyacrylamide, 7M urea) and staining with 0.2% methylene blue.

Example 2: Antisense Polynucleotide Effects on Smooth Muscle Cell Proliferation In Vitro

The proliferation of smooth muscle cells was studied in vitro in the presence of antisense polynucleotides directed against rodent or human tenascin mRNA. Antisense polynucleotides having the sequences set

forth in SEQ ID NOs:7-20 were synthesized in accordance with the procedures of Example 1. The effects of those antisense polynucleotides were studied using the procedures outlined below.

A. Smooth Muscle Cell Isolation and Culture

Male Sprague-Dawley rats weighing 350-450 g were euthanized with carbon dioxide. The carotid arteries were removed and trimmed free of adventitia, nerve, and fat under a dissecting microscope. Arteries were cut into approximately $1mm^3$ pieces and placed in a 125 ml Erlenmeyer flask containing 0.67 ml/carotid artery in the following enzyme cocktail: 79.2 ml Hanks Balanced Salt Solution (Gibco; HBSS), 0.8 ml 0.2M $CaCl_2$ (Fisher), 0.286 g HEPES free acid (Calbiochem), 0.03 g trypsin inhibitor (Sigma; Type I-S; 10,000 units/mg), 0.16 g bovine serum albumin (Sigma; Fraction V), 600 units elastase (Sigma; Type II-A; 28 units/mg), 16,000 units collagenase (Worthington; CLS II; 353 units/mg) adjusted to pH7.4, and 0.2μm filtered.

The flask was placed on an orbital shaker at 150 rpm at 37°C for 2-2.5 hr. The suspension was triturated vigorously and filtered through a 70 μm nylon cell strainer. The filtrate was then centrifuged at 400 x g for 10 min. The pellet was resuspended in 4 ml/carotid artery in the following media: 20% fetal bovine serum albumin (Hyclone; FBS); 2mM glutamine (Gibco); 100 units/ml penicillin G sodium (Gibco); 100 μ/ml streptomycin sulfate (Gibco); DMEM (Gibco). The cell suspension from one carotid artery was then seeded into one T25 flask (Falcon) and maintained at 37°C in 5% CO_2.

B. Proliferation Assay

After 6-7 days, cells were rinsed twice with PBS (phosphate buffered saline) and harvested by the addition of 4 ml of 0.05% trypsin-

EDTA (Gibco; 0.25% trypsin-EDTA) followed by incubation at 37°C for 3-5 min. The flask was rinsed with an additional 4 ml media (DMEM, 20% PBS, 2 mM glutamine, 50 units/ml penicillin, 50 µg/ml streptomycin). The trypsinized cells and the rinse were combined and centrifuged at 400 x g for 10 min.

The supernatant was removed and 5 mls of fresh media was added to the pellet. The pellet was resuspended by vigorous trituration, and the number of cells was determined using a Coulter counter.

The cells were diluted to 3,500 cells/100 µl and, using a 12 channel digital micropipette seeded (100 µl/well) in a 96 well (Falcon) flat-bottom, microtiter cell culture plate. The culture plate was then incubated at 37°C in 5% CO_2.

The following day, each well was rinsed twice with 100 µl PBS, and overlaid with 100 µl/well growth arrest media: 0.1% FBS (heat inactivated at 65°C for 45 min.); 2mM glutamine; 50 units/ml penicillin; 50 µg/ml streptomycin. Four days later, the growth arrest media was removed. The cell number was determined (treatment day counts) using a Coulter counter by averaging the cell number from three wells.

To the remaining wells was added 100 µl complete media (DMEM, 10% FBS/65°C inactivated, glutamine, pen/strep) without or with various antisense polynucleotides. The plates were placed in an incubator at 37°C in 5% CO_2.

Three days later, the wells were rinsed twice with 100 µl each PBS. The cell number from 3 wells was again determined (assay day counts) using a Coulter counter. To the remaining wells was added 100 µl

of 45 μg/ml Calcein A-M (Molecular Probes) in PBS. The plates were incubated for 1 hr. at 37°C. After incubation, fluorescence was determined using a Cytofluor 2350 (Millipore) microtiter plate reader with excitation at 485 nm and emission at 530 nm. Growth in cell number was calculated by subtracting treatment day cell counts from assay day cell counts. Based on the established linear relationship between fluorescence and cell number, the percent growth inhibition by the antisense polynucleotides was determined.

Where antisense polynucleotides directed against human mRNA were used, the proliferation assay employed sections of human aorta in place of rat artery as described above.

The results of these studies are summarized in Figure 1 and Table 1. The data in Figure 1 are depicted to indicate that 100% inhibition reflects the absence of smooth muscle cell proliferation during the assay. The data in Figure 1 show that 50μM of antisense polynucleotide, SEQ ID NO:7, showed nearly 90% inhibition of smooth muscle cell proliferation while 10μM showed about 35% inhibition.

Table 1

SEQ ID NO	% Growth Inhibition
7	45
8	38
9	36
10	41
11	48
12	50
13	53
14	39
15	62
16	44
17	49
18	41
19	55
20	57

The data clearly show that all tested antisense polynucleotides significantly inhibited smooth muscle cell proliferation. The data also show that a process of the present invention is effective in humans as well as non-human species.

Example 3. Antisense Polynucleotide Effects on Smooth Muscle Cell Proliferation In Vivo

The following studies were performed to demonstrate the efficacy of a process of the present invention in inhibiting smooth muscle cell proliferation in vivo. These studies were performed using various antisense polynucleotides having the sequences set forth in SEQ ID NOs:7-20. Those antisense polynucleotides were prepared in accordance with the procedures in Example 1.

A Balloon Angioplasty Model

Balloon angioplasty of the rat carotid artery was performed as previously described by Clowes *et al.* (Lab Invest 1983, 49:327-333). Briefly,

male Sprague-Dawley rats weighing 375-425g were anesthetized. A 2F embolectomy catheter was then inserted into the left iliac artery and advanced to the distal end of the left carotid artery. The balloon was inflated and pulled down the artery 3 times. The catheter was then removed.

A second similar catheter that lacked a tip was filled with either an antisense molecule, or with a pharmaceutically acceptable carrier, and was attached to a syringe pump. The catheter was then inserted into the left iliac artery and advanced into the left carotid artery.

Antisense polydeoxyribonucleotides (1mM in DMEM) directed against tenascin mRNA, PAI1 mRNA or carrier alone (DMEM alone) were delivered at 6μl/min for 5 min, with the catheter tied to the proximal portion of the artery to prevent blood form flowing around the catheter tip and washing the delivered material out of the artery. The carotid artery was then ligated distal to the heart, near the bifurcation of the internal and external branches of the artery. After 15 min of static incubation, the ligatures and the catheter were removed to restore normal blood flow. Fifty μl of antisense polynucleotides, or carrier, was then applied to the adventitial surface of the carotid artery.

Two weeks after this treatment, the animals were sacrificed, perfuse-fixed with 10% formalin, and the central portion of the carotid artery was embedded in paraffin, according to standard protocols. Five μm sections were then stained with hematoxylin and eosin, again according to standard protocols.

Neointimal and medial areas were measured and the intima/media ratio calculated. The ratio for the treatment groups was then

normalized to the ratio for the control groups. As seen in Figure 2, antisense polynucleotide having the sequence of SEQ ID NO:13 inhibited intimal thickening by approximately 58%, while the nonspecific control antisense polynucleotide to PAI1 (GGCTGAAGAC ATCTGCAT (SEQ ID NO:66)) had essentially no effect. These data show that an antisense polynucleotide of the present invention specifically inhibits neointimal development in vivo in the rat carotid balloon angioplasty model of restenosis.

B. Overexpression of Tenascin mRNA After Angioplasty

The overexpression of tenascin mRNA was tested using the rat carotid artery balloon angioplasty model of restenosis. Abnormal proliferation of smooth muscle cells often leads to restenosis in humans; these experiments were designed to determine whether tenascin mRNA is overexpressed in response to angioplasty.

At various times after balloon angioplasty of rats, arteries were removed from the anesthetized animals, trimmed of adventitia and nerve tissue, and mRNA levels were determine by reverse transcriptase - polymerase chain reaction (RT-PCR), according to standard procedures in the art.

Glyceraldehyde phosphate dehydrogenase (GAPDH) mRNA levels were also determined as an internal control for these experiments. GAPDH is a housekeeping gene whose expression would be expected to remain constant in the tested cells.

Results from these studies are shown on Table 2, below.

Table 2
Time Course of mRNA Expression in Response to
Balloon Angioplasty of Rat Carotid Artery

	Time Post-Angioplasty												
mRNA	control	2hr	4hr	6hr	1d	2d	4d	7d		10d		14d	
								int	med	int	med	int	med
GAPDH	++	++	++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
Tenascin	-	-	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++
PCNA	-	-	-	+	+++	++++							++

Values represent relative band intensities of PCR amplified products on gels.

As the data in Table 2 show, GAPDH levels remained fairly constant. PCNA mRNA levels were determined in order to assess the proliferation of neointimal and medial smooth muscle cells.

As further shown in Table 2, tenascin mRNA was not detectable in control, non-ballooned arteries. Expression was induced to a maximum level between 4 hours and 14 days post-angioplasty. As indicated by the expression levels of PCNA mRNA, this time frame correlates with the proliferation of smooth muscle cells.

Smooth muscle cells were then isolated from rat arteries 14 days after angioplasty according to the technique described above. Cells were grown in culture for approximately 3 weeks. Expression levels of tenascin, GAPDH and PCNA mRNAs were then determined by RT-PCR and the results are shown in Table 3.

Table 3

mRNA	Neointima	Media
GAPDH	+++++	+++++
Tenascin	++	++++
PCNA	++++	+++

Values represent relative band intensities of amplified products on gels.

Tenascin mRNA was overexpressed in both neointimal and medial smooth muscle cells in vitro, with greater overexpression in the medial smooth muscle cells. Thus, the altered in vivo genotype observed in the arteries was maintained in tissue culture. As the expression levels of PCNA mRNA show, neointimal cells were also more actively proliferating than were medial cells.

C. Antisense-mediated Down Regulation of Target mRNA

The specificity, efficacy and mechanism of action of antisense polynucleotides can be examined by studying the down-regulation of target mRNA by such antisense polynucleotides. In these experiments, growth arrested smooth muscle cells from the rat carotid artery were serum-stimulated in the presence or absence of 50μM antisense polynucleotide designated SEQ ID NO:13. After 3 days of contact with the antisense polynucleotide, total RNA was isolated from the smooth muscle cells and RT-PCR was performed. The results from these studies are shown in Table 4 below.

Table 4

Antisense Treatment	% Growth Inhibition	mRNA	Days of Serum Stimulation 0	3
None	0	GAPDH	++++	++++
None	0	Tenascin	+	+++
SEQ ID NO:13	75	GAPDH	++++	++++
SEQ ID NO:13	75	Tenascin	+	++
Vinculin	18	GAPDH	++++	++++
Vinculin	18	Tenascin	+	+++

Table 4 shows that SEQ ID NO:13 markedly inhibited the induction of its target mRNA, but had no effect on the control housekeeping gene, GAPDH. An antisense polynucleotide directed against vinculin (CGTATGAAAC ACTGGCAT (SEQ ID NO:65)) did not affect the induction of mRNA from the tenascin gene or expression of the GAPDH gene. These data show that the growth inhibition by antisense polynucleotides directed against the tenascin gene are the result of specific inhibition of the production of tenascin.

The foregoing specification, including the specific embodiments and examples is intended to be illustrative of the present invention and is not to be taken as limiting. Numerous other variations and

modifications can be effected without departing from the true spirit and scope of the present invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: ANTISENSE MOLECULES DIRECTED AGAINST A TENASCIN GENE

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(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/037,025
(B) FILING DATE: 25-MAR-1993

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TGGCARYAGC YRRGTCAYGG CCCCCATGGT GGAGGT 36

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ACCTCCACCA TGGGGGCCRT GACYYRGCTR YTGCCA 36

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CYRRGTCAYG GCCCCCAT 18

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

ATGGGGGCCR TGACYYRG 18

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CYRRGUCAYG GCCCCCAU 18

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

AUGCCCCGGR UGACYYRG 18

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CCAGGTCACG GCCCCCAT 18

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GGCCCCCATG GTGGAGGT 18

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TGGCAGTAGC CAGGTCAC 18

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TAGCCAGGTC ACGGCCCCCA T 21

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CAGTAGCCAG GTCACGGCCC CCAT 24

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CCAGGTCACG GCCCCCATGG T 21

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TAGCCAGGTC ACGGCCCCCA TGGT 24

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CTGAGTCATG GCCCCCAT 18

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CAGCTGAGTC ATGGCCCCCA TGGT 24

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CTGAGTCATG GCCCCCATGG T 21

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CAACAGCTGA GTCATGGCCC CCAT 24

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CAGCTGAGTC ATGGCCCCCA T 21

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CTGAGTCATG GCCCCCATGG TGGA 24

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CAACAGCTGA GTCATGGCCC CCATGGT 27

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

ATGGGGGCCG TGACCTGG 18

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

ACCTCCACCA TGGGGGCC 18

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GTGACCTGGC TACTGCCA 18

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

ATGGGGGCCG TGACCTGGCT A 21

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

ATGGGGGCCG TGACCTGGCT ACTG 24

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

ACCATGGGGG CCGTGACCTG G 21

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

ACCATGGGGG CCGTGACCTG GCTA 24

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

ATGGGGGCCA TGACTCAG 18

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

ACCATGGGGG CCATGACTCA GCTG 24

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

ACCATGGGGG CCATGACTCA G 21

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

ATGGGGGCCA TGACTCAGCT GTTG 24

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

ATGGGGGCCA TGACTCAGCT G 21

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

TCCACCATGG GGGCCATGAC TCAG 24

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

ACCATGGGGG CCATGACTCA GCTGTTG 27

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

CCAGGUCACG GCCCCCAU 18

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GGCCCCCAUG GUGGAGGU 18

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

UGGCAGUAGC CAGGUCAC 18

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

UAGCCAGGUC ACGGCCCCCA U 21

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) IENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

CAGUAGCCAG GUCACGGCCC CCAU 24

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

CCAGGUCACG GCCCCCAUGG U 21

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

UAGCCAGGUC ACGGCCCCCA UGGU 24

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

CUGAGUCAUG GCCCCCAU 18

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

CAGCUGAGUC AUGGCCCCCA UGGU 24

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

CUGAGUCAUG GCCCCCAUGG U 21

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

CAACAGCUGA GUCAUGGCCC CCAU 24

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

CAGCUGAGUC AUGGCCCCCA U 21

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

CUGAGUCAUG GCCCCCAUGG UGGA 24

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

CAACAGCUGA GUCAUGGCCC CCAUGGU 27

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

AUGGGGGCCG UGACCUGG 18

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

ACCUCCACCA UGGGGGCC 18

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

GUGACCUGGC UACUGCCA 18

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

AUGGGGGCCG UGACCUGGCU A 21

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

AUGGGGGCCG UGACCUGGCU ACUG 24

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

ACCAUGGGGG CCGUGACCUG G 21

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

ACCAUGGGGG CCGUGACCUG GCUA 24

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

AUGGGGGCCA UGACUCUG 18

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

ACCAUGGGGG CCAUGACUCA GCUG 24

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

ACCAUGGGGG CCAUGACUCA G 21

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

AUGGGGGCCA UGACUCAGCU GUUG 24

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

AUGGGGGCCA UGACUCAGCU G 21

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

UCCACCAUGG GGGCCAUGAC UCAG 24

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

ACCAUGGGGG CCAUGACUCA GCUGUUG 27

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: mRNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

ACCUCCACCA UGGCGGCCRU GACYYRGCUR YUGCCA 36

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

TGGCARYAGC YRRGTCAYGG CCCCCATGGT GGAGGT 36

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

CGTATGAAAC ACTGGCAT 18

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

GGCTGAAGAC ATCTGCAT 18

WHAT IS CLAIMED IS:

1. A synthetic antisense polynucleotide of less than about 50 bases comprising a nucleotide sequence that is identical to at least 18 contiguous bases of SEQ ID NO:1.

2. The polynucleotide of claim 1 wherein the bases of said polynucleotide are linked by pseudophosphate bonds that are resistant to cleavage by exonuclease or endonuclease enzymes.

3. The polynucleotide of claim 2 wherein said bonds are phosphorothioate bonds.

4. The polynucleotide of claim 1 comprising the nucleotide sequence shown in SEQ ID NO:3.

5. The polynucleotide of claim 1 having the sequence of SEQ ID NO:7-20, 35-47 or 48.

6. A synthetic antisense polynucleotide of less than about 50 bases comprising a nucleotide sequence that is identical to at least 18 contiguous bases of SEQ ID NO:2.

7. The polynucleotide of claim 6 wherein the bases of said polynucleotide are linked by pseudophosphate bonds that are resistant to cleavage by exonuclease or endonuclease enzymes.

8. The polynucleotide of claim 7 wherein said bonds are phosphorothioate bonds.

9. The polynucleotide of claim 6 comprising the nucleotide sequence shown in SEQ ID NO:4.

10. The polynucleotide of claim 6 having the sequence of SEQ ID NO:21-34, 49-61 or 62.

11. A pharmaceutical composition comprising the polynucleotide of claim 1 or claim 6 and a physiologically tolerable diluent.

12. A process of inhibiting vascular smooth muscle cell proliferation comprising inhibiting the expression of tenascin in said vascular smooth muscle cell.

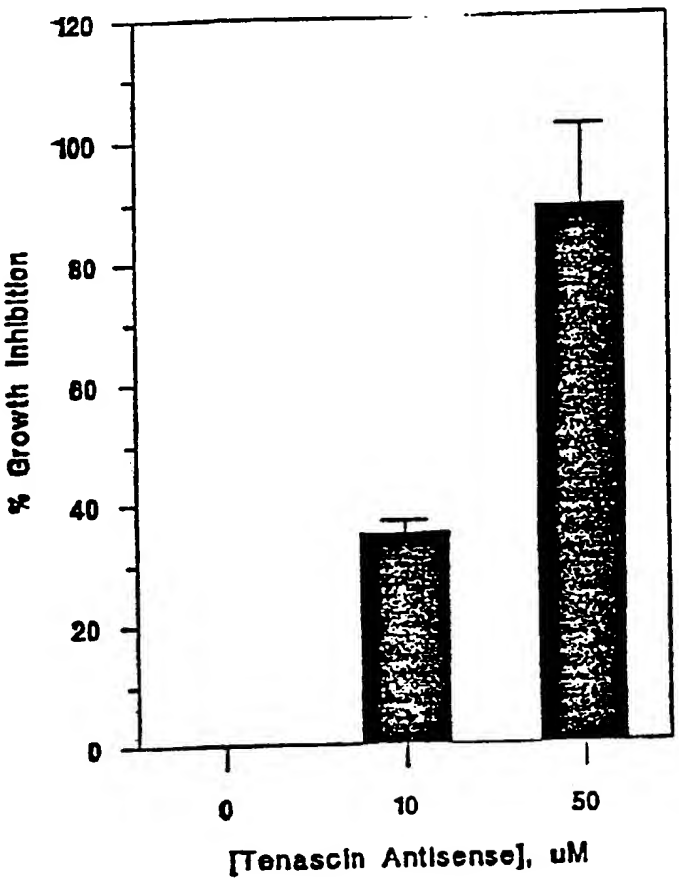
13. The process according to claim 12 wherein inhibiting the expression of tenascin is inhibiting the transcription of the gene that encodes tenascin.

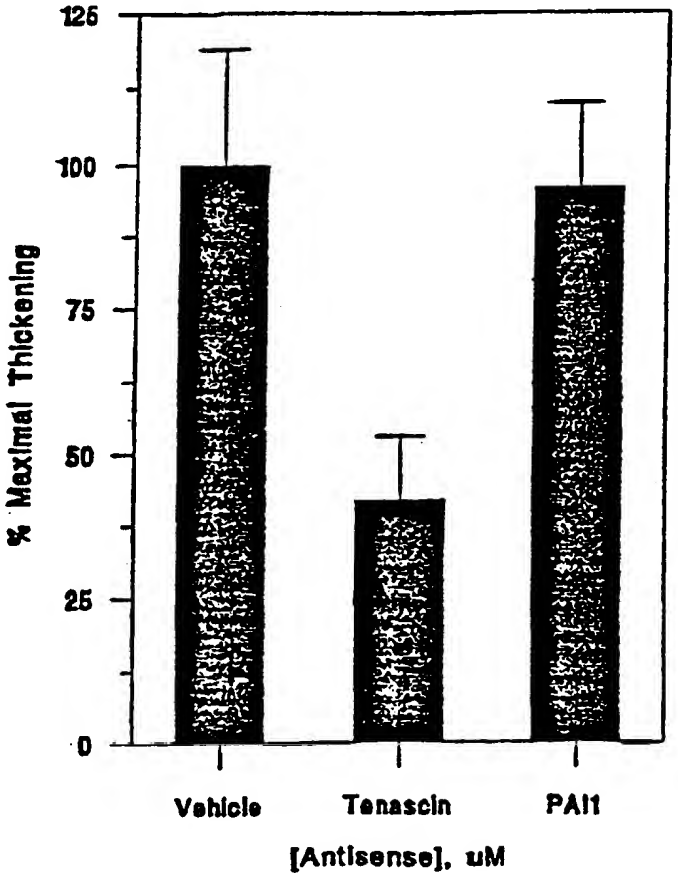
14. The process according to claim 13 wherein inhibiting the transcription of the gene that encodes tenascin is accomplished by exposing said smooth muscle cell to an antisense polynucleotide of less than about 50 bases comprising a nucleotide sequence that is identical to at least 18 contiguous bases of SEQ ID NO:1.

15. The process according to claim 12 wherein inhibiting the expression of tenascin is inhibiting the translation of mRNA that encodes tenascin.

16. The process according to claim 15 wherein inhibiting the translation of mRNA that encodes tenascin is accomplished by exposing said smooth muscle cell to an antisense polynucleotide of less than about 50

bases comprising a nucleotide sequence that is identical to at least 18 contiguous bases of SEQ ID NO:2.


120
100
80
60
40
20
0
% Growth Inhibition
0
10
50
[Tenascin Antisense], uM


125
100
75
50
25
0
% Maximal Thickening
Vehicle
Tenascin
PAI1
[Antisense], uM

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/03206

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C07H 21/04; A61K 48/00
US CL :536/24.5, 24.31; 514/44;
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/24.5, 24.31; 514/44;

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, BIOSIS
search terms: antisense, tenascin

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Journal of Cell Biol., Volume 112, Number 2, issued January 1991, Weller et al., "Amino acid sequence of mouse tenascin and differential expression of two tenascin isoforms during embryogenesis", pages 355-362, see abstract.	1-16
Y	Journal of Biol. Chem., Volume 266, Number 27, issued 25 September 1991, Chiang et al., "Antisense oligonucleotides inhibit intercellular adhesion molecule 1 expression by two distinct mechanisms", pages 18162-18171, see abstract.	1-16

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
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Date of the actual completion of the international search 20 MAY 1994	Date of mailing of the international search report 07 JUN 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer KENNETH R. HORLICK Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/03206

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
Y	Proc. Natl. Acad. Sci. USA, Volume 88, issued September 1991, Agrawal et al., "Pharmacokinetics, biodistribution, and stability of oligodeoxynucleotide phosphorothioates in mice", pages 7595-7599, see abstract.	1-16